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Efficient Synthesis of Ribonucleotide Reductase Inhibitors 3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (3-AP) and 3-Amino-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (3-AMP) *via* Palladium Mediated Cross-Coupling Reactions

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Abstract: An efficient synthesis of potent ribonucleotide reductases inhibitors 3-amino-pyridine-2-carboxaldehyde thiosemicarbazone (3-AP) and 3-amino-4-methyl-pyridine-2-carboxaldehyde thiosemicarbazone (3-AMP) is described. The synthesis of 3-AP and 3-AMP was achieved in 4 and 5 steps, with overall yields of 61% and 39%, respectively. The synthesis featured a convergent approach utilizing a Stille coupling strategy to prepare vinylpyridine derivatives. A more economic way to synthesize vinylpyridine using Heck reaction was also discussed.

Ribonucleotide reductases exist in all living cells and catalyze the rate-limiting step in the synthesis of deoxyribonucleoside triphosphates. Inhibitors of ribonucleotide diphosphate reductases are extremely effective in blocking the biosynthesis of DNA because of the low intracellular levels of deoxyribonucleoside triphosphates.[1] Efforts have been devoted to the design and synthesis of strong inhibitors of ribonucleotide diphosphate reductases which would serve as particularly useful therapeutic agents against cancer. Several classes of of ribonucleotide diphosphate reductase inhibitors have been developed, including hydroxyurea,[2] N-heterocyclic carboxaldehyde thiosemicarbazones (HCTs),[3] nonapeptides,[4] polyhydroxy-benzohydroxazones[5] and nucleoside analogs[6]. Among these known inhibitors, hydroxyurea and gemcitabine are the only compounds in clinical use for the treatment of cancer. The HCTs are among the most potent known inhibitors of ribonucleoside diphosphate reductase being 80-5000 times more potent than hydroxyurea *in vitro*. Recently, Sartorelli and co-workers developed several new HCTs that by virtue of their structure are resistant to rapid metabolic clearance[7]. Among them, the most active compounds were 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) and 3-amino-4-methylpyridine-2-carboxaldehyde thiosemicarbazone (3-AMP). These compounds showed significant antitumor activity in mice bearing L1210 leukemia, M-109 lung carcinoma, and A2780 human ovarian carcinoma. This activity has led to the selection of 3-AP for clinical development providing the impetus for the development of a convergent synthesis of these agents. Herein we wish to report efficient syntheses of both 3-AP and 3-AMP.

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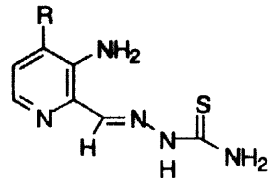


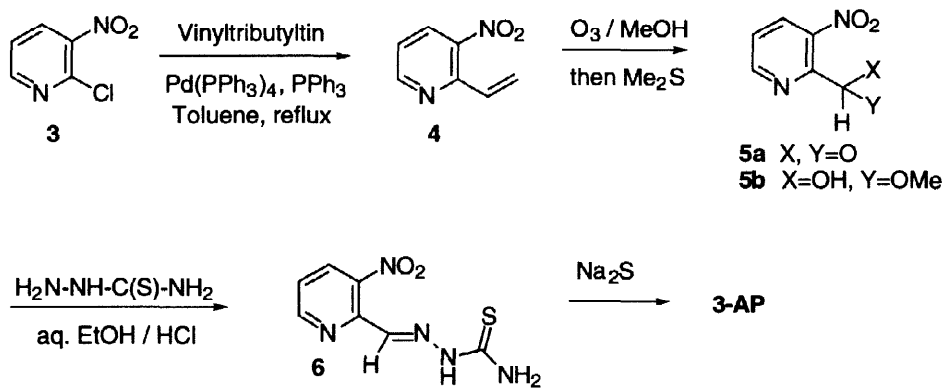
1, R=H, 3-AP
2. R=CH_3, 3-AMP

Figure 1

Results and Discussion

The discovery and synthesis of 3-AP and 3-AMP were reported recently by Sartorelli and co-workers at Yale[7b]. However, low overall yields for these two compounds (9.3% for 3-AP and 2.3% for 3-AMP) were reported because of an inefficient sequence leading to the formation of 3-nitropyridine-2-carboxaldehyde and 4-methyl-3-nitropyridine-2-carboxaldehyde respectively. Encouraged by the significant antitumor activity of 3-AP and 3-AMP, we initiated a synthetic program with the aim of providing multi-gram quantities of the compounds for further preclinical study.

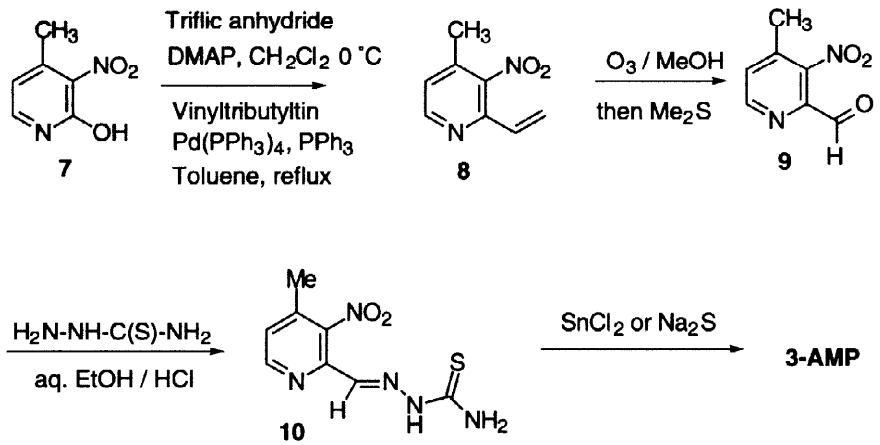
Scheme 1



With the aim of efficiently introducing the 2-carboxaldehyde function and avoiding the tedious reaction sequence in the original synthesis, a Stille coupling reaction was designed to provide the vinylpyridine **4** through a cross-coupling reaction of the halogenated pyridine with vinyltin[8] as outlined in Scheme 1. To avoid protection of the amino group, the nitro was carried through the synthesis until the last step, where it was reduced to the amino group. Thus, the coupling of 3-nitro-2-chloropyridine with vinyltributyltin occurred smoothly in the presence of 5% tetrakis(triphenylphosphine) palladium [$Pd(PPh_3)_4$] and triphenylphosphine, and gave **4** in 86% isolated yield. We found that the amount of $Pd(PPh_3)_4$ could be reduced to 1% without compromising the yield.

Ozonolysis of the vinyl group to the corresponding aldehyde was initially performed in methylene chloride, which resulted in complex products and low yield. Replacing methylene chloride with methyl alcohol or a mixed solvent consisting of methyl alcohol/methylene chloride afforded a mixture of the desired pyridine-2-carboxaldehyde and its hemiacetal (**5a and 5b**) in excellent yield[9]. Coupling of the aldehyde and hemi-acetal (**5a and 5b**) with thiosemicarbazone was achieved by using slightly modified literature procedure to give **6** in 95% yield[7b]. The nitro group in **6** was reduced by excess Na_2S in ethanol at reflux to give **3-AP** in 82% yield[10]. Thus, the synthesis of 3-AP was accomplished in 4 steps with 61% overall yield, which represented a 6-fold improvement over that of the original synthesis.

Scheme 2

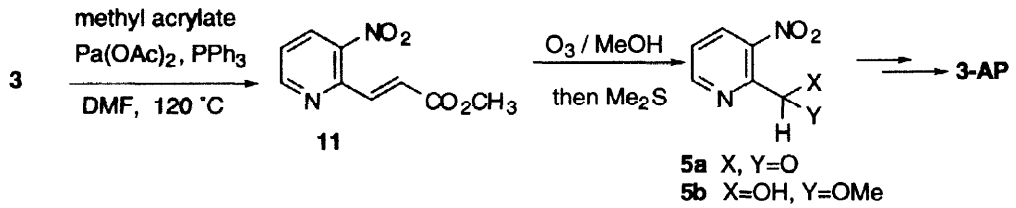


We then turned our attention to the synthesis of 3-AMP. Following a similar strategy to that used for the synthesis of 3-AP (Scheme 1) a Stille coupling reaction between the aromatic triflate and vinyltributyltin was employed for the introduction of the vinyl group in **8** [8]. Treatment of 2-hydroxy-4-methyl-3-nitropyridine in methylene chloride at 0 °C with triflic anhydride in the presence of 4-dimethylaminopyridine offered the corresponding triflate in 87% yield. The resulting triflate was reacted with vinyltributyltin using 5% tetrakis(triphenylphosphine) palladium as a catalyst in the presence of triphenylphosphine, and afforded the desired 2-vinyl-substituted pyridine **8** in 69-76% yield [11]. The remaining steps of the synthesis were similar to that described in the synthesis of 3-AP (Scheme 1) with the exception of the last step, where the reduction of nitro group in **10** was performed with excess $SnCl_2$ in ethanol at reflux to give 3-AMP in 65% yield[12]. The whole synthesis was completed in 5 steps with an overall yield of 39.1%, which was 17 fold improved over the previously reported synthesis of 3-AMP [7b].

Alternatively, an intermolecular Heck reaction could also be used to introduce a vinyl group as shown in Scheme 3 [13]. The reagents used for the Heck reaction (methyl acrylate and catalyst palladium acetate) were much cheaper than those used in Stille reaction, although the unoptimized yield of **11** was moderate (51%). Compound **11** was then ozonized to give the desired 2- pyiridine-2-carboxaldehyde and its hemi- acetal (**5a**

and 5b), which merged with the previous synthesis of 3-AP as shown in Scheme 1. Further investigation of syntheses of 3-AP and 3-AMP using Heck reaction will be reported in due course.

Scheme 3



In summary, we have presented efficient syntheses of 3-AP and 3-AMP which were based on the Stille coupling reaction to provide key intermediates, the vinyl pyridine derivatives **4** and **9**. The overall yields for preparation of 3-AP and 3-AMP are much higher than that of reported in the literature. It is worth noting that the initial result of using the Heck reaction to generate vinyl pyridine **12** also represents an economical way to synthesize 3-AP.

Experimental Section

Proton and carbon NMR spectra were recorded at 300 MHz and 75 MHz, respectively, and are reported in ppm with tetramethylsilane as internal standard. Electron impact mass spectra were measured at a 70-eV ionizing voltage. Chemical ionization spectra were obtained using methane or isobutane as the reagent gas, fast atom bombardment spectra were obtained in a magic matrix. All reagents were purchased from Aldrich Co. and used as received.

2-Vinyl-3-nitropyridine (4). A toluene solution (15 mL) of 2-chloro-3-nitropyridine (417 mg, 2.63 mmol), $Pd(PPh_3)_4$ (32 mg, 0.026 mmol), triphenylphosphine (20 mg, 0.078 mmol) and vinyltributyltin (1.00 g, 3.16 mmol) was heated to reflux for 2 hr. The reaction mixture was cooled to room temperature and then quenched with water (10 mL). The resulting mixture was extracted with EtOAc (3X30 mL). The combined organic layers were dried (Na_2SO_4) and filtered. The filtrates were conc. *in vacuo* and the residue was purified by silica gel chromatography (20-30% EtOAc/Hexanes) to provide 339 mg (86%) of the desired product **4** as a pale yellow oil. ^1H NMR (300 MHz, $CDCl_3$): δ 8.75 (dd, J=1.3 Hz, 4.5 Hz, 1H), 8.16 (dd, J=1.3 Hz, 8.1 Hz, 1H), 7.23-7.36 (m, 2H), 6.59 (dd, J=1.6 Hz, 16.7 Hz, 1H), 5.70 (dd, J=1.6 Hz, 10.4 Hz, 1H).
LRMS calcd. for $C_7H_7N_2O_2$ (MH^+): 151, found: 151.

2-Carboxaldehyde (5a) and 2-Hemiacetal (5b). A methanol solution (20 mL) of vinylpyridine **4** (800 mg, 5.33 mmol) was subjected to ozonolysis at -78°C for 15 min. The reaction was quenched at -78°C with Me_2S

(2.2 mL), and the resulting reaction mixture was stirred overnight at room temperature. After solvent was evaporated, the residue was subjected to a short silica gel column, providing 850 mg (95%) of a mixture of products **5a** and **5b** (**5a/5b** = 3:2). The ^{1}H NMR spectrum of aldehyde **5a** matches the reported literature data [Ref. 7(a)]. ^{1}H NMR of hemiacetal **5b** (300 MHz, $CDCl_3$): δ 8.78 (m, 1H), 8.34 (m, 1H), 7.57 (m, 1H), 6.10 (d, J=8.7 Hz, 1H), 5.53 (m, 1H), 3.47 (s, 3H).

Compound (6). To an aqueous ethanol solution (7 mL of ethanol and 3 mL of water) of aldehyde **5a** and hemiacetal **5b** (0.286 g, 0.178+0.108 mmol) was added conc. HCl (2 drops) at room temperature, followed by thiosemicarbazide (0.24 g, 2.2 mmol). The reaction mixture was stirred at r.t. for 7 hr. The resulting yellow solids were collected via filtration. The solids were rinsed sequentially with water (40 ml), ethanol (40 ml) and ether (50 ml), and then dried under high vacuum, affording 0.36 g (90%) of the desired product **6**: ^{1}H NMR (300 MHz, DMSO-d6): δ 11.97 (s, 1H), 8.85 (dd, *J* =1.5 Hz, 5.1 Hz, 1H), 8.60 (s, 1H), 8.40 (dd, *J* =1.2 Hz, 6.9 Hz, 1H), 8.27 (s, 1H), 7.67 (dd, *J* =4.8 Hz, 8.4 Hz, 1H), 7.09 (s, 1H).). ^{13}C NMR (75 Mhz, DMSO-D6): δ 178.8, 152.4, 145.2, 144.2, 137.8, 132.2, 124.7. HRMS (FAB) calcd. for $C_7H_7N_5O_2S$ (M+1): 225.0320, found: 225.0320.

3-Aminopyridine-2-carboxaldehyde Thiosemicarbazone (3-AP). An aluminum foil covered flask was filled with hydrazone compound **6** (112.5 mg, 0.5 mmol) and EtOH/H_2O (10 ml, 1:1). To this mixture was added $Na_2S•9H_2O$ (480 mg, 2.0 mmol) and stirred for 20 h at rt. The resulting solution was separated into two equal portions: The majority of the solvent of the first portion was removed via vacuum and the residue was acidified with HCl (0.5 M) to pH 7. The mixture was filtered and the solid product was rinsed with H_2O (2 ml), EtOH (2 ml), and Et_2O (2 ml). The resulting product was dried under high vacuum to give 35 mg (72%) of **3-AP**. For the second portion, most of the solvent was removed and the residue was purified by silica gel column to offer 40 mg (82%) of **3-AP.** ^{1}H NMR (300 MHz, DMSO-d6): δ 11.29 (s, 1H), 8.31 (s, 1H), 8.15 (br, 1H), 7.95 (br, 1H), 8.27 (s, 1H), 7.80 (dd, *J* =1.2,4.2Hz, 1H), 7.07 (m, 2H), 6.43(br, 2H). ^{13}C NMR (75 Mhz, DMSO-D6): δ 177.0, 149.0, 144.0, 137.1, 132.7, 124.4, 122.3. LRMS (CI, NH_3) 196 (M+1), HRMS (FAB) calcd for $C_7H_{10}N_5S$ (M+1): 196.0657, found 196.0657.

4-Methyl-3-nitro-2-vinylpyridine (8). To a mixture of 2-hydroxy-3-nitro-4-methylpyridine (3.08 g, 20 mmol) and 4-dimethylaminopyridine (2.44 g, 20 mmol) in 5 ml of methylene chloride was slowly added triflic anhydride (5.7 g, 21 mmol) at 0 °C. The reaction mixture was stirred at 0 °C overnight, then was diluted with 200 mL of CH_2Cl_2, followed by washing with water and brine, dried over $MgSO_4$. After removing the solvent , the crude compound was chromatographed on a silica gel column, using 50 % ethyl acetate in hexane to provide 4.95 g (87%) of the triflate.

A mixture of 2-hydroxy-3-nitro-4-methylpyridine triflate thus prepared (7.74 g, 27.06 mmol), tributyl vinyl tin(10.3 g, 32.47 mmol) and tetrakis(tripheylphosphine) palladium(0) (1.56 g, 1.35 mmol) in 100 mL of anhydrous toluene was heated to refluex for 3 hr, then cooled to room temperature and quenched with 20 mL of brine. The mixture was then extracted with ethyl aceatate (3 x 100 ml) and the combined organic layer was dried over $MgSO_4$. After evaporating the solvent, the crude product was purified by a silica

gel column (hexane : ethyl acetate=5 : 1) to provide 2.82 g (70%) of the desired compound **8**. ^{1}H-NMR ($CHCl_3$): δ 8.51 (d, *J* =5.1 Hz, 1H), 7.13 (d, *J* =5.1 Hz, 1H), 6.66 (dd, J=10.5 Hz, 12.3 Hz, 1H), 6.55 (d, J=1.8 Hz, 1H), 5.63 (dd, J=10.5 Hz, 1.8 Hz, 1H), 2.32 (s, 3H). HRMS calcd for $C_8H_8N_2$ 164.0586, found 164.0586.

4-Methyl-3-nitropyridine-2-carboxaldehyde (9). A solution of **8** (4.03 g, 24.54 mmol) in 100 ml of methanol was ozonolyzed at -78°C and the reaction was monitored by TLC. After the reaction, the excess O_3 was removed by bubbling the reaction mixture with O_2 at -78 °C for 5 min. The reaction was then quenched at -78°C with Me_2S (10 mL), and the resulting reaction mixture was stirred overnight at room temperature. The solvent was evaporated, the residue was purified by silica gel chromatography, (Hexanes: EtOAc =4:1 to Hexanes: EtOAc =2:1) to provide 3.51 g (86%) of aldehyde **9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.02 (s, 1H), 8.76 (d, J=4.8 Hz, 1H), 7.54 (d, J=4.8 Hz, 1H), 2.42 (s, 3H). ^{13}C NMR (75 Mhz, DMSO-D6), d 189.2, 150.5, 142.4, 140.6, 130.1, 16.2.

Compound (10). To a mixture of aldehyde **9** (3.11 g, 18.73 mmol) in 20 mL of 70% aqueous ethanol was added thiosemicarbazide (6.36 g, 1.45 mmol) in 70 ml of 70% aqueous ethanol at room temperature. The reaction mixture was stirred at r.t. for 8 h. The resulting yellow solids were collected via filtration and rinsed sequentially with water (40 ml), ethanol (40 ml), ether (50 ml), and then dried under high vacuum, affording 4.12 g (92%) of the desired product **10**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.01 (s, 1H), 8.72 (s, 1H), 8.66 (d, J=4.8 Hz, 1H), 7.58 (d, J=4.8Hz, 1H), 6.67 (s, 1H), 2.30 (s, 3H). ^{13}C NMR (75 Mhz, DMSO-D6): δ 178.8, 150.5, 142.3, 137.1, 139.6, 137.7, 126.4, 15.7. HRMS calcd. for $C_8H_9N_5O_2S$ (M+1): 240.0555, found: 240.0557.

3-Amino-4-methylpyridine-2-carboxaldehyde Thiosemicarbazone (3-AMP). To a solution of $SnCl_2 \bullet 2H_2O$ (1.5 g, 31.4 mmol) in 20 ml of EtOH was added hydrazone compound **10** (1.5 g, 6.28 mmol). The resulting mixture was heated to reflux for 24 h and then cooled to rt., filtered, the filtrate was evaporated and the residue was dissolved in 20 ml of water. The resulting solution was adjusted to pH 7.5 with sat. $NaHCO_3$ and extracted with THF (3x50 ml). The combined organic phases were dried over Na_2SO_4 and concentrated in vacuum. The resulting residue was purified on a silica gel column (CH_2Cl_2/MeOH/NH_4OH, 100:7:0.5) to give 0.185 g (14%) of 3-AMP; The solid from filtration was dissolved in 100 ml of hot water and filtered again. The filtrate was adjusted to pH 7.5 with sat. $NaHCO_3$, stirred at rt for 30 min., filtered and the solid product was rinsed with H_2O (20 ml), EtOH (10 ml), and Et_2O (50 ml). The resulting product was dried under high vacuum to give an additional 0.653 g (50%) of 3-AMP. The combined yield of 3-AMP was 0.938 g (64%). ^{1}H NMR (300 MHz, DMSO-d6): δ 11.33 (s, 1H), 8.34 (s, 1H), 8.17 (br, 1H), 7.93 (br, 1H), 7.76 (d, *J* =4.4Hz, 1H), 6.99 (d, *J* =4.4Hz, 1H), 6.16(br, 2H), 2.16 (s, 3H). ^{13}C NMR (75 Mhz, DMSO-D6): δ 177.2, 149.4, 142.4, 137.3, 132.3, 130.6, 125.4, 16.9. FABMS (M+1) 210.

Compound (11). A mixture of 2-chloro-3-nitropyridine (1.20 g, 7.6 mmol), methyl acrylate (1.31 g, 15.2 mmol), triethylamine (0.92 g, 9.1 mmol), triphenylphosphine (0.60 g, 2.28 mmol) $Pd(OAc)_2$ (0.17 g, 0.76

mmol), and 15 ml of DMF in a sealed tube was heated to 120 °C for 24 hr. The reaction mixture was cooled to room temperature and then quenched with water (10 mL). The resulting mixture was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4 and filtered. The filtrates were conc. *in vacuo*, and the residue was purified by silica gel chromatography (Hexane: EtOAc=4:1) to provide 0.80 g (51%) of the desired product **11** as yellow solid. ^1H NMR (300 MHz, $CDCl_3$): δ 8.85 (dd, J=1.5 Hz, 4.5 Hz, 1H), 8.16 (dd, J=1.5 Hz, 8.1 Hz, 1H), 7.49 (dd, J=4.8, 8.4 Hz, 1H), 7.22 (d, J=15.3 Hz, 1H), 3.85(s, 3H). LRMS calcd. for $C_7H_7N_2O_2$ (MH^+): 209, found: 209. HRMS calcd for $C_9H_8N_2O_4$ (M+1): 208.0484, found 208.0484.

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